

A BIOCHEMICAL INVESTIGATION OF THE CORPUS LUTEUM

by

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## I. INTRODUCTION.

Notwithstanding the large volume of literature dealing with the theory as an organ of internal secretion, our knowledge of the subject is still fragmentary. The relationship between the state of development of the ovary and the activity of the vagina remains at present obscure.

The writer wishes to express his sincere gratitude to Professor F.A.E. Crew, to Dr B.P. Wiesner, and to Dr J.M. Robson, for much valuable help, advice and criticism during the progress of this work.

It is hoped that this volume will be useful to those engaged in the study of internal secretion.

It is intended to give a brief account of the various aspects of the subject, dealing more fully with the work on the internal secretion and the ovary, and to present the results which have been obtained during the course of the writer's study.

## II. SUMMARY.

From the very earliest days it has been known that the removal of the gonads results in very profound



## I. INTRODUCTION.

Notwithstanding the large volume of literature dealing with the ovary as an organ of internal secretion, our knowledge of the subject is still fragmentary. The relationship between the state of development of the ovary, and the activity of the vagina, uterus and mammary glands, immediately suggests that the relationship is mutual or a one sided dependency. Oophorectomy and transplantation experiments (in the early days) and administration of ovarian extracts (more recently) have made it clear that this control is exerted by the ovary through the agency of internal secretions.

It is intended to give a brief account of the various aspects of the subject, dealing more fully with the work on the corpus luteum, and thereafter to present the results which have been obtained during the course of the writer's study.

### (1) Oophorectomy.

From the very earliest days it has been known that the removal of the gonads results in very profound/

profound changes in the organism. When the operation is performed prior to puberty, the effects are particularly striking; the accessory organs of reproduction remain in an infantile condition, and the cyclic changes of oestrus fail to make their appearance. Post-pubertal oophorectomy results in the immediate cessation of all cyclic activity and a gradual atrophy of the vagina, uterus and mammary glands. Amongst those who have worked in this field may be mentioned the following:- Bucura (18), Carmichael and Marshall (21), Marshall and Jolly (80), and Halban (48).

That the effects are due to a disturbance of the uterine vascular supply (Hofmeir,<sup>59</sup>) or nervous connections (Sokoloff,<sup>100</sup>) is out of the question, since many workers have taken specific precautions against such possible effects.

## (2) Transplantation.

The evidence of transplantation experiments gives further proof of the two fundamental points:-

(a) that the ovary controls the activity of the accessory reproductive organs, and (b) that the control is exerted by means of internal secretions.

Many workers - Marshall and Jolly (80), Knauer (63),<sup>79</sup>  
Halban/

Halban (48), Carmichael (20), Ribbert (94 ), Limon (70), Grigorieff (47), Rubenstein (97 ), - have demonstrated that the atrophy of the accessory reproductive organs, which normally occurs after oophorectomy, does not take place if an ovarian graft is made, and further that there is a continuance of the oestrus cycle even although the ovary be transplanted on to the abdominal wall or peritoneum. Morris (81), Glass (44), Dudley (34) and Cramer (31) have described cases of women suffering from disturbances of menstruation having the disorder corrected by ovarian implant. Wiesner (109) reports experiments in which intra-uterine grafts were so far successful that conception, followed by normal pregnancy, was the result.

### (3) Ovarian Extracts.

In no other branch of the study of the ovary, from the point of view of the chemist, has there been more indifferent work than in the preparation of ovarian extracts, and it is from this line that the ultimate proof of the endocrine nature of the ovary must come. The work so briefly outlined above required the final link to complete the chain of evidence/

evidence, and it is only within recent years that any real progress has been made.

It is clear that there are three functions of the ovary performed by means of internal secretions:-

1. Development of the accessory reproductive organs.
2. Cyclic changes associated with oestrus.
3. Changes of pregnancy or pseudo-pregnancy.

The question arises: Does the ovary perform these three functions by means of one and the same autacoid?

(a) Oestrus-producing extracts.

The first type of extract - a saline extract described by Jentzner and Beuttner (62), and later by Bucura (17) appears to have been ineffective; Marshall and Jolly (79), however, report oestrus changes in the anoestrus bitch by injection of saline extracts of ovaries. On the other hand, injection of liquor folliculi (Sonnerberg, 101) was without effect or possibly the interpretation of the results faulty. Iscovesco (61), in 1912, prepared an extract by means of organic solvents which produced hypertrophy of the uteri of adult animals. Fellner (39) seems to have been the first to have used oophorectomised animals for such experiments, and he/



he showed that the injection of lipoid extracts of ovaries and placentae produced the changes characteristic of oestrus.

During the last decade a tremendous amount of work has been done. The Allen-Doisy technique for the determination of oestrus has facilitated the investigation of the activity of extracts, and has made the results more dependable. Allen and Doisy( 5) Brouha and Simonnet (15), Butenandt(19), Courrier(28), Dodds(1 ), Doisy, Veler and Thayer(33), Laqueur(69), Lipschutz(71), Loeb(75 ), Loewe(76 ), Marrian (78 ), Papanicolaou( 86), Parkes and Bellerby( 88), and Zondek(116), are some of the principal workers who have described the preparation of an oestrus producing extract from various sources.

A variety of names has been proposed for this oestrus-producing substance which has a wide distribution both in animal tissues, body fluids and in plants. 'Ovarian' or 'Sex' hormone presuggests that only one exists; 'Folliculin' implies elaboration by the follicle only; 'Oestrin' that its function is limited to the production of oestrus which/



which is not the case (a point to be discussed later). Since it is doubtful whether all the changes occurring during oestrus are induced by this substance, it seems undesirable to suggest any such exclusive specificity and therefore Wiesner and Crew (11) propose the less committal name 'alpha factor'.

The abundance of alpha in the body fluids during pregnancy is one of the most difficult problems requiring explanation. There is some evidence (Weichert, 10) that the preliminary action of alpha is necessary for the effective action of the luteal hormone, and in this case the presence of alpha during pregnancy may be a necessary complement to the action of the corpus luteum. Certainly the idea of a balance between alpha and the corpus luteum is a necessary assumption. The source of alpha during pregnancy is another question requiring elucidation. Fellner (40) suggests that the ovarian interstitial tissue is responsible for its elaboration, while the occurrence of alpha in large quantities in the placenta led many workers - Allen (2) and Aschheim (11) in particular - to conclude that elaboration of the hormone is carried on in this organ.

Since it has been shown that injection of alpha/

alpha during pregnancy leads to abortion or re-absorption, its secretion by the placenta would thus be a definite anomaly. It seems reasonable to suppose that the placenta absorbs alpha from the maternal circulation in order to protect the developing foetus from its action.

During the last two years a great deal of work has been done on the chemistry of alpha. Many workers have described the preparation of crystalline, water-soluble 'oestrin', but until chemical and physical data are forthcoming it cannot be said that all the preparations described are identical substances. Marrian(78) isolated an active crystalline compound for which preliminary analysis and molecular weight determinations suggested the formula  $C_{18}H_{24}O_3$ ; its melting point was  $263-265^{\circ}$ . Doisy, Veler and Thayer(33) claimed to have prepared the hormone in a crystalline form, and gave as its formula  $C_{18}H_{21}(OH)_2$  and melting point  $249^{\circ}$ . Butenandt(19) recently described the preparation of an active crystalline compound and gave as the formula  $C_{18}H_{22}O_2$ , i.e. differing from that given by Marrian by  $H_2O$ . The activity of these pure preparations is of the order of 8,000,000 mouse units per/

per gram. Marrian's crystals (and in most respects those of other workers) are characterised by a fairly low solubility in organic fat solvents. The presence of the phenolic grouping -  $C_6H_5(OH)$  - is shown by the strong positive reactions given in Millon's test and the xanthoproteic reaction. It dissolves slowly in potassium hydroxide from which it is immediately precipitated by carbon dioxide. The iodine value showed the presence of one double bond,  $-C=C-$ , as also did bromination. Marrian prepared an acetate and showed the presence of three hydroxyl groups; the physiological activity of the acetate was considerably lower than that of the hydroxy compound. Butenandt has prepared a crystalline compound having the same formula as Marrian's crystals, and has succeeded in converting it into  $C_{18}H_{22}O_2$  by heating it with potassium hydrogen sulphate.

In view of the fact that at least two different chemical compounds exist, both of which induce oestrus changes, it is possible that several 'oestrins' exist. On the other hand it seems unlikely that oestrus-producing activity could be an intrinsic property of two or more different molecules, and it may be that the substances isolated are/

are inactive and merely associated with minute amounts of the real active principle.

(b) Corpus Luteum.

During pregnancy or pseudo-pregnancy the cyclic changes of oestrus are held in abeyance, and there is a marked development of the mammary glands and uterine endometrium. As early as 1897 Beard (13) and Prenant(92) suggested that the corpus luteum was the responsible agent, and this hypothesis has been borne out by experiment, at least in part, in more recent years.

It has been shown that the claims of Aschheim, Zondek and other workers, who regarded alpha as 'the ovarian hormone' responsible for the sex cycle, were unjustified. Alpha was shown to produce only the first phase of the cycle but not the phenomena of pregnancy or pseudo-pregnancy. Wiesner(110), Corner and Allen(25), Asdell and Marshall (12), Courier and Masse(30), Loeb and Kountz(75) and Ebhardt(35) demonstrate that alpha produces none of the uterine reactions characteristic of early pregnancy. It seems certain therefore that the corpus luteum contains a hormone or hormones which are entirely distinct from alpha in their effects.

To/



To the corpus luteum has been attributed the role of 'ductless gland of pregnancy', and to it the various phenomena, occurring in the accessory organs of reproduction during gestation, have been relegated. The phenomena for which the corpus luteum may be responsible are as follows, and will be dealt with in turn.

- i. Proliferation of the uterine endometrium.
- ii. Sensitisation of the uterus.
- iii. Inhibition of the reaction of the uterus to pituitrin.
- iv. Inhibition of oestrus and ovulation.
- v. Development of the mammary glands.
- vi. Relaxation of the pubic ligaments.
- vii. Maintenance of pregnancy.

Most of the work reported in this thesis has been carried out on the rabbit in which animal oestrus persists in the absence of copulation, and in which ovulation is not spontaneous but requires the stimulus of coitus (Heape, 53).

After sterile copulation, the rabbit exhibits a well defined pseudo-pregnant period lasting for 14 days, during which changes occur in the uterus similar to those found in the first half of pregnancy. Ovulation in the rabbit takes place some ten hours after copulation; certain changes occur/



occur in the ruptured follicle which result in the formation of the corpus luteum.

i. Proliferation of the uterine endometrium.

After sterile copulation in the rabbit and the consequent formation of corpora lutea, the uterus undergoes a marked hypertrophy - vascularisation and particularly glandular increase - similar to that seen in the early stages of pregnancy. During this pseudo-pregnant period a cross section of the uterus presents a fern-like appearance, and it has been shown by Ancel and Bouin(8) that ablation of the corpora lutea prevents these changes.

Corner(23), Corner and Allen(25), Allen(3), Patel(90), Robson and Illingworth(96) et al. have described the preparation of corpus luteum extracts which, when injected into mature oophorectomised rabbits, have the property of bringing about this special histological and physiological state of the uterine mucosa characteristic of early pregnancy. For the substance responsible for these changes, Allen(3), proposes the name 'progestin'; Wiesner and Patel(113) investigating similar extracts propose the name 'kythin' - (pregnancy hormone). Since the substance responsible for the production of progestational proliferation may not bring about  
all/

all the changes occurring during pregnancy, and on the other hand, may have actions other than those related to pregnancy, Wiesner and Crew(111) suggest the noncommittal term 'beta' which name shall be used throughout this thesis.

ii. Sensitisation of the uterus.

Gustav Born seems to have been the first to suggest that the corpus luteum was responsible for the nidation of the fertilised ovum, and the classical experiments of Fraenkel(41) carried out at the suggestion of Born, showed that, if the ovaries of a rabbit were removed from one to six days after coitus, pregnancy terminated prematurely in abortion or the non-attachment of the embryo. Ancel and Bouin( 6) confirmed this work also in the rabbit.

Loeb(72) originally showed that the uterus in a post-oestrus condition reacted to mechanical irritation by the production of placentomata, and that this sensitivity was entirely dependent on the presence of corpora lutea. Corner and Warren (27) were able to produce the same reaction in the rat during lactation.

In the normal unmated cycle of the rat there is no pseudo-pregnant period and Evans and Long(38) found/

found that in this animal no response to mechanical irritation was shown unless pseudo-pregnancy had been induced by sterile copulation. Hammond (49) and Nielsen (83) have confirmed these results by inducing deciduometa formation in rabbits during pseudo-pregnancy. Teel (106) and Brouha (14) by injection of alkaline extracts of the anterior lobe of the pituitary have caused luteinisation of the ovary and report that the uterus of the rat under these conditions, responds in the same way. Weichert (108) Nelson and Pfiffner (82) and Goldstein and Tatelbaum (46) have shown that injection of corpus luteum extracts also causes this sensitisation.

It may be assumed that progestational proliferation is only the physiological expression of sensitisation.

iii. Inhibition of the reaction of the uterus to pituitrin.

It is an established fact that the uterus of a normal non-pregnant animal shows remarkable spontaneous activity and gives a contraction with pituitrin in vivo. In consequence it is frequently used for standardising drugs. This reaction of the uterus to pituitrin is a general reaction of all smooth muscle, and, from the point of view of the work to be/

be reported upon shortly, is particularly important.

In the rabbit, after copulation, certain changes occur in the ovary which result in the formation of the corpus luteum. At the same time important changes take place in the uterine endometrium - progestational proliferation etc. - and the uterus no longer shows any response to pituitrin. Some mechanism must come into play which has the effect of 'neutralising' those factors responsible for uterine contractions. Such must be the case since the continuance of pregnancy would be impossible were the uterus to retain its excitability to pituitrin. After sterile copulation, when a period of pseudo-pregnancy ensues, the uterus exhibits similar phenomena to those occurring during pregnancy. The return of the excitability of the uterus to pituitrin at the end of pseudo-pregnancy and pregnancy which, in the latter coincides with parturition, corresponds to regression of the corpus luteum. It has been shown by Knaus (65) that the injection of an extract of the corpus luteum into a non-pregnant rabbit inhibits the reaction of the uterus to pituitrin; the activity of the extract was determined by its power to bring/



bring about progestational proliferation of the uterus as described by Corner and Allen(25).

iv. Inhibition of ovulation and oestrus.

Hammond(51) in the cow, and Loeb (73) and Papanicolaou (85) in the guinea pig have shown that ablation of the corpora lutea expedites the appearance of the next oestrus, whereas abnormal persistence of the corpus luteum, whether pathological or experimental, results in the suppression of oestrus - Ochsner(84). Hess(55), Williams(114), and Tandler(104) Loeb(74). Much work has been done with extracts of the corpus luteum with a view to suppressing oestrus, and varying results reported. Inhibition of oestrus has been reported by the following: Macht, Stickels and Seckinger(77), Brouha and Simonnet(16), Parkes and Bellerby (89), Payne Cartland and Van Peenan (91), Hisaw Meyer and Weichert(58), Haterius and Pfiffner(52) and Patel(90) whereas Corner and Hurni (27) failed to achieve this result. Frank and Gustavson (43), Iscovesco(60), Glimm and Wadehn(45) and Herrmann(54) all claim to have obtained an oestrus producing extract from the corpus luteum; it seems clear, however, from the work of Parkes and Bellerby (89) and from the author's own/



own experience that alpha is not found in solid corpora lutea and that its presence in corpus luteum extracts is due to the use of cystic corpora.

The fact that ablation of corpora lutea induces oestrus suggests that the ovary is, in some species, continuously under a tonus of ovulation, and that the periodic appearance of oestrus is due to the periodic involution of the corpus luteum. However there are other factors which must be taken into account.

Since cornification of the vaginal epithelium is taken as the criterion of oestrus, it must be regarded as an effect of alpha upon the vaginal epithelium, and since the secretion of alpha by the ovary is known to be under the influence of anterior pituitary factors, a chain of reactions may be written thus:

$$A \rightarrow \rho \rightarrow O \rightarrow \alpha \rightarrow V \rightarrow C$$

where A = anterior pituitary,  $\rho$  = oestrogenic substance, O = ovary,  $\alpha$  = alpha, V = vaginal epithelium and C = cornification. Consequently inhibition of oestrus may be brought about by interrupting this chain at any of its links. Corpus luteum extract then might inhibit cornification by

(1) acting upon the vaginal epithelium and preventing its reaction to alpha; (2) by preventing the reaction of the ovary to the pituitary stimulus; or finally (3) by inhibiting the pituitary function and thus preventing the production of the oestrogenic stimulus. According to Patel, oestrus is prevented by the inhibition of alpha secretion by the ovary 'whose reactivity to the oestrogenic stimulus is reduced' and 'it is probable that 'kythin' also acts on the pituitary, inhibiting the secretion of oestrogenic substance'. Patel further states that there is no chemical 'neutralisation' of alpha by the inhibitor substance. It is difficult to reconcile this statement with the vast amount of alpha found during pregnancy in, for example, mare's urine.

v. Development of the mammary glands.

The growth and development of the mammary glands may be divided into four stages:-

1. Pre-pubertal,
2. Pubertal.
3. Pseudo-pregnancy.
4. Pregnancy.

That the development of the mammary glands is endocrine in nature is beyond doubt since mammary tissue, when transplanted to abnormal sites, will continue its normal development (Ribbert, 94 ).

Obviously/

Obviously the first two stages of development cannot be due to any influence of the corpus luteum since the first oestrus occurs before the ovary has ever contained any corpora; since the growth recurs at each oestrus it has been suggested by Herrmann (54), Fellner(39), Ancel and Bouin ( 6), Frank (42) et al. that the stimulus is alpha. Laqueur (68) by injection of very large doses of alpha in the guinea pig induced mammary growth with lactation which he was able to maintain by continued injections of small doses. Hammond (50) and Ancel and Bouin (10) have studied mammary development in the rabbit in considerable detail, and these authors describe the proliferation which occurs during pseudo-pregnancy and pregnancy. By removal of the corpora lutea formed after sterile copulation, Ancel and Bouin ( 9 ) showed that no such development took place.

Various sources of the stimulus for the 3rd and 4th stages have been suggested - foetus, placenta and ovary - but from the work of Parkes (87) it is assumed that the corpus luteum is the responsible agent. By means of alkaline extracts of the anterior pituitary, Parkes was able to prolong pseudo-pregnancy to the length of pregnancy (rabbit) and he claims/

claims to have obtained a state of development of the mammary glands equivalent to that at parturition. It would thus appear that no foetal or placental factors are required, and that the development is entirely dependent on the corpus luteum.

Turner and Frank (107) maintain that for complete development, it is necessary to inject simultaneously, corpus luteum extract together with increasing amounts of alpha. In direct contradiction of Parkes' work comes that of Stricker and Greuter<sup>102</sup>(~~103~~) and Corner(24) By injection of potent extracts of corpus luteum - the potency being judged by their power to bring about progestational proliferation - Corner was unable to induce mammary growth; also by causing ovulation (and consequent formation of corpora lutea) by the injection of urine of pregnant women, Corner was again unable to obtain mammary growth. He then injected alkaline extracts of anterior pituitary into oophorectomised virgin rabbits, and found complete development of the mammary gland with lactation. Stricker and Greuter arrived at a similar conclusion, but they maintain that the animal must have been under the influence of corpora lutea at some time shortly before the injection of the kyogenic extracts, thus implying that the corpus luteum exerts a more or less lasting preparatory effect/



effect on the mammary gland. Until this work has been repeated and extended further, discussion appears profitless.

vi. Relaxation of the pubic ligaments.

56,  
Hisaw (57) has described the preparation of a corpus luteum extract from corpora of the sow, which has the remarkable property of dissolving the pubic ligaments of the guinea pig. This extract also has the property of inhibiting oestrus and inducing uterine sensitivity to mechanical irritation.

Since corpora lutea of the cow contain no such relaxative principle, this phenomenon cannot be regarded as being one of general importance.

vii. Maintenance of pregnancy.

As already pointed out there can be little doubt that the corpus luteum is necessary for the sensitisation of the uterus and those other phenomena characteristic of pregnancy.

In the rabbit removal of the ovaries during pregnancy is inevitably followed by the abortion or reabsorption of the foetuses (Hammond, 50 ).

Allen and Corner ( 4 ) report that if female rabbits/



rabbits are mated and oophorectomised 18 hours later, while the ova are still in the Fallopian tube, corpus luteum extract (progestin) substitutes for the removed ovaries so completely that the embryos are nourished, become implanted, and develop in the uterus exactly as in normal pregnancy under the influence of the mother's own corpora lutea.

It follows that the corpus luteum produces substances which are responsible for the phenomena occurring during pregnancy, and it remains to be determined whether or not one or more hormones are elaborated.

(4) Relation of the anterior pituitary to ovarian activity.

Smith and Engle (99) , and Zondek and Aschheim (115) have shown that the prepuberal ovary of the immature animal can be activated by grafts of anterior lobe substance. Evans (37) on the other hand, reported that alkaline extracts of anterior pituitary inhibit the oestrus cycle in mature animals. Laqueur (67) claimed that the urine of pregnant women contained substances which inhibit/

inhibit ovulation and oestrus.

The difference in the effects following injection of Evans' extracts and the injection of macerated fresh tissue, led to the tentative supposition that two different anterior pituitary principles were involved.

Wiesner and Crew (111) refer to these gonadotropic factors as Rho 1 and Rho 2, and assume that Rho 1, or a mixture of Rho 1 and Rho 2 with a preponderance of the former, incites the elaboration of alpha by the ovary. Rho 2, then is the kyogenic factor and is responsible for the incitement of beta production.

Dickens (32), Wiesner and Marshall (112) describe the preparation of Rho factors and their purification from urine of pregnancy.

Object/

Object of the Investigation.

Knaus assumed that the inhibition of the reaction of the uterus to pituitrin was caused by the hormone of the corpus luteum which causes the morphological changes of the uterus during pregnancy (beta factor).

This author (65) showed, however, that during the first half of pregnancy in the rabbit, the uterus is practically inactive owing to loss of contractility, while during the second half there occurs a continuous rise in spontaneous activity which reaches a climax at parturition. It should be noted that the corpus luteum remains active throughout the whole duration of pregnancy.

It was thus necessary to determine whether the factors responsible for the progestational and inhibitory reactions were really identical, and experiments have been performed to investigate in more detail the relation between these uterine phenomena.

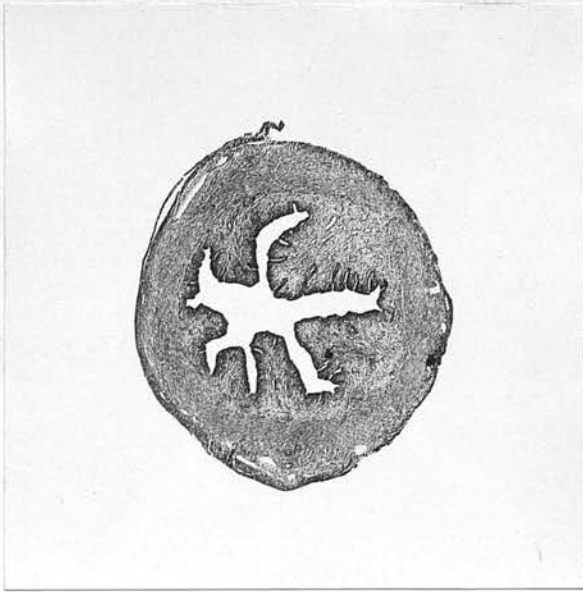


Plate 1. Section of the uterus of a normal rabbit.

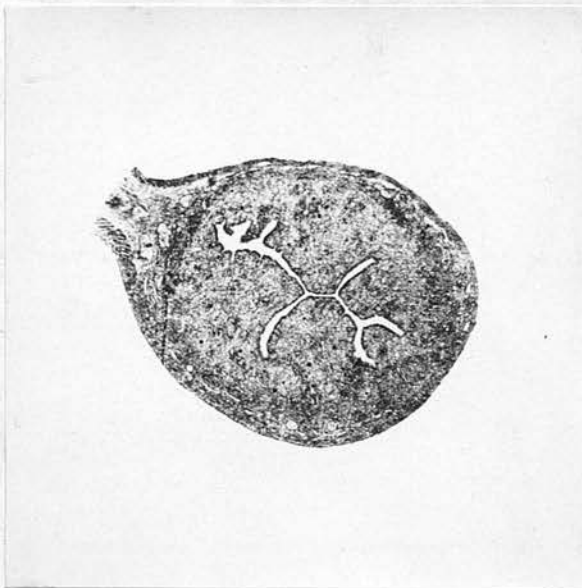


Plate 2. Section of the uterus of a rabbit after  
oophorectomy. (From Marshall's 'Physiology of  
Reproduction')





Plate 3. Section of the uterus of a rabbit, injected with  
corpus luteum extract showing proliferation ++

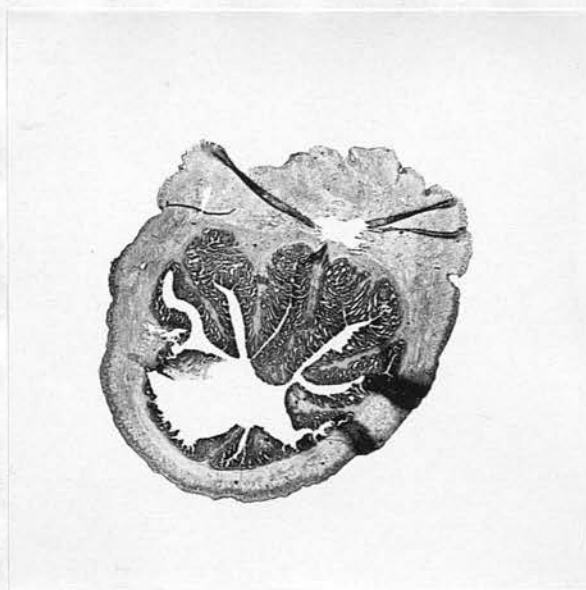


Plate 4. Section of the uterus of a rabbit, injected with  
corpus luteum extract, showing proliferation +++

## II. EXPERIMENTAL.

### Methods of Extraction.

The methods of preparation of ovarian extracts in general, and corpus luteum extracts in particular, of the majority of workers seem to be based on the same systematic scheme, designed first to extract the lipoids together with the active substance or substances, and thereafter to remove the inactive material by differential solubilities, fractional distillation, etc.

Hisaw extracts corpora lutea of the sow with acid alcohol, adjusts to neutrality with dilute sodium carbonate, evaporates in vacuo, dissolves the residue in ether and precipitates the phosphatides with four volumes of acetone. The acetone-ether mixture when evaporated, yields a fraction which causes prompt relaxation of the ligamenta pubium when administered to female guinea pigs, and in addition produces other phenomena characteristic of early pregnancy, such as inhibition of oestrus and sensitisation of the uterine mucosa.

Parkes/

Parkes and Bellerby use corpora lutea of the cow and their mode of preparation is as follows:- corpora are minced and dehydrated with anhydrous sodium sulphate; the dry paste is then extracted thoroughly in the cold with ether, the ether extract evaporated to small bulk and the phosphatides precipitated with acetone. The acetone soluble fraction is a dark brown oil which on injection inhibits oestrus.

Allen describes the preparation of an extract which causes progestational proliferation of the rabbit's uterus. Minced corpora of the sow are extracted with boiling ethyl alcohol (95%) several times; the alcohol is removed by vacuum distillation and the residue extracted with ether. The ether is evaporated and the phosphatides removed with acetone. This acetone soluble fraction constitutes Allen's crude extract. Further purification is carried out as follows:- The fats and cholesterol are removed by freezing from methyl alcohol, and the remaining traces of cholesterol by partitioning between 70% ethyl alcohol and petroleum ether.

Knaus employs the same mode of preparation for his extracts which in addition to causing progestational proliferation, also inhibits the reaction of the uterus to pituitrin.

Standard/

Standard Extract.

The method used in the preparation of corpus luteum extracts in this research was as follows:- Cow's ovaries were received from the Glasgow abattoirs within a few hours of the animals being killed; the corpora were dissected out and any cystic glands carefully washed with water to remove the fluid which is comparatively rich in alpha. The solid tissue was then minced and extracted at least twice with 95 per cent. ethyl alcohol in the cold for 24 hours each extraction. The alcohol was then filtered off and removed under reduced pressure at a temperature not exceeding 45°C.

Some considerable difficulty was experienced with this distillation owing to the high water content of the alcohol and consequent foaming. However, by the use of a large Claissen flask with the side tube bent at an angle of about 45°, it was found possible to remove the alcohol with the minimum of inconvenience at the rate of about 1000 c.c. per hour. Care was taken to prevent overheating when the solvent was nearly all removed.

The residue so obtained from this alcohol extraction was then extracted with 300 c.c. portions of methylated ether until the ether remained colourless.

The/



The tissue left after the alcohol extraction was spread in a thin layer and dried in an air oven with a strong current of warm air; when quite dry, it was thoroughly extracted with ether in a Soxhlet apparatus until no more colouring matter remained. The two ether extracts were then combined and the ether removed by distillation under reduced pressure. The dark brown viscous oil remaining was extracted with ethyl acetate, in which a large amount of inactive fatty material was insoluble, until the ethyl acetate remained colourless. On removing the ethyl acetate by vacuum distillation a reddish brown oil was obtained which solidified on standing. The density of this crude extract averaged about 0.8 gm. per c.c. There was considerable variation in the yield, but on the average 25 gm. per kilo. of fresh corpora was obtained.

Each preparation was tested and the activity determined. In the majority of cases, 1 c.c. of such an extract produced distinct progestational proliferation and inhibition of the reaction of the uterus to pituitrin, when injected into an oophorectomised rabbit in five daily doses of 0.2 c.c. each. It had been found also (by Dr J.M. Robson of this laboratory) that the injection of an effective/

effective amount of the corpus luteum extract (1 - 1.5 c.c.) in six doses at 12 hourly intervals (the animal being killed 12 hours after the last injection) inhibited the in vitro reaction of the uterus to pituitrin. Section of the uterus showed that proliferation had been induced also. Injections over 48 hours (at 12 hourly intervals) were also effective in causing inhibition, but the degree of proliferation was comparatively small. As it was desired to investigate both the progestational and inhibitory reactions in the same animal, the longer period of injection was chosen for the majority of the experiments.

In this research 80 mature female non-pregnant rabbits weighing about 2 kilos. each were used. The animals were of various breeds, as it was found extremely difficult to obtain rabbits of the same breed in sufficient numbers. The animals were kept under standard conditions of feeding and temperature, and it can be stated that the results obtained were not due to variations occurring between different breeds of animals.

Bilateral oophorectomy was performed in each animal on the day prior to the beginning of the injections as follows:- A mature female non-pregnant/

The animals were killed by a blow on the occiput or by injection of 10 c.c. air into an ear vein. One horn of the uterus was placed in Bouin's solution for histology, and the other used for the experiments in vitro. Ringer-Locke solution was used in all cases in 100 c.c. containers. Temperature (37.5°C.) and oxygen bubbling were maintained constant and records of uterine contractions taken on smoked drums with lightly balanced levers.

The supplies of pituitrin, pitressin and pitocin were obtained from Messrs Parke Davis, Ltd. to whom the writer is greatly indebted. The contractility of the uterus was in all cases tested by the subsequent addition to the bath of 0.1 c.c. of 1:1000 adrenalin solution.

Various attempts to purify the crude extracts were made from time to time, but these will be referred to as separate experiments.

During the progress of the work it was noticed that occasionally the extracts varied in their ability to bring about both progestational proliferation and inhibition of the pituitrin reaction, although the mode of preparation of the extracts was adhered to strictly. Extracts were obtained which gave one without the other. As will be seen from Table/

Table I, Beta 20, 21, 22, 23, 25, 29 and 35 were below standard, i.e. these extracts were lacking in their ability to cause both proliferation and inhibition of the pituitrin reaction. Beta 20 and 25 were prepared exclusively from cystic ovaries, the fluid of which was carefully removed. In view of experiments to be reported upon shortly, the possible presence of alpha can hardly explain the results obtained. Since comparatively few cystic corpora were obtained from each batch of ovaries received, some time elapsed before sufficient tissue was collected. It was found that storage in alcohol destroyed the activity of the corpora and this will be considered later when discussing beta 29. Beta 21 showed complete inactivity. This preparation was carried out in the normal way except that corpora lutea of the sow were used. This extract remained oily but in other respects resembled the standard extracts. No explanation can be offered for its inactivity. Beta 22 and 23 were prepared from corpora lutea received during the early summer. The appearance of the corpora was pale and since the original mode of extraction was adhered to, it can only be assumed that the glands contained less active material than usual. Beta 29 will be discussed/



discussed later.

The remaining extracts were normal in that progestational proliferation and complete inhibition followed their injection into oophorectomised rabbits. Thus the two parallel effects were demonstrated.

Table I.

Extract No.	Wt. of tissue in gm.	Animal No.	Proliferation	Inhibition.
β 10	800	Ra 12	+++	++
11	700	21	+++	++
12	700	25	+++	++
15	1000	59	++++	++
18	700	76	+++	++
20	600	118	+++	+
21	1100	109	-	-
22	700	122	++	++
23	700	158	+++	+
24	600	163	+++	++
25	800	181	++	-
29	900	224	++	-
30	500	238	+++	++
35	700	242	+++	-

Good proliferation = +++

Fair do. = ++

Complete inhibition = ++

Partial inhibition = +

Administration/

Administration of Known Doses of Alpha together with  
Corpus Luteum Extracts.

In a number of experiments known doses of alpha (prepared from pregnancy urine) were added to the corpus luteum extract injected, and the pro-gestational and pituitrin reactions of the uteri of the injected animals determined. It was found that small doses of alpha did not affect either of the actions of the corpus luteum extracts. The addition of 1000 mouse units of alpha to the corpus luteum extract injected into one animal, appeared to decrease slightly the proliferation reaction, but the response of the uterus to pituitrin was still absent. When larger doses (more than 3000 mouse units) of alpha were used in one animal, the pro-liferative reaction was no longer produced, whereas the uterus, greatly enlarged and hyperaemic, still showed no response to pituitrin. When similar doses of alpha alone were injected, the response of the uterus to pituitrin was not affected.

Experiment: Ra 27 received 6 injections at 12 hourly intervals of 0.2 c.c. beta 10 + 200 m.u. alpha each injection. Section of the uterus 12 hours after the last injection showed decreased proliferation...++

The/

The response to pituitrin was absent.

Ra 35 received 6 injections at 12 hourly intervals of 0.2 c.c. beta 12 + 600 m.u. alpha each injection. Section of the uterus 12 hours after the last injection showed muscular hypertrophy, but no glandular proliferation. The in vitro reaction of the uterus to pituitrin was completely inhibited.

Ra 20 and Ra 28 received 800 and 1800 m.u. alpha total dose respectively. In neither animal was there any sign of progestational proliferation, nor was the response of the uterus to pituitrin affected.

#### Injection of fluid from an ovarian cyst.

While these experiments were in progress the fluid, removed at operation from an ovarian cyst of a patient suffering from disturbances of menstruation, was received from Dr Young. 10 c.c. of the fluid was injected within 5 days into an oophorectomised rabbit and caused no progestational proliferation as shown by section of the uterus. The response of the uterus to pituitrin was, however, almost absent. The injection of 0.2 c.c. of the fluid into three oophorectomised mice produced no cornification of the vagina.

This experiment, together with those already described/

described, gave further indication that the factor in the corpus luteum responsible for progestational proliferation might be different from the substance inhibiting the reaction of the uterus to pituitrin and an attempt was made to obtain two such substances separately. The methods employed will now be described.

Investigation of alcohol, and of ether extracts.

Corpora lutea of the cow were extracted in the normal manner with alcohol (95 per cent.) twice in the cold for 24 hours each extraction. The alcohol was removed under reduced pressure and the residue extracted thoroughly with ether. The ether was evaporated, and the brown viscous oil, beta 13A, injected. Ra 43 received 0.25 c.c. (one quarter of the total extract, equivalent to 150 gm. fresh corpora lutea) in maize oil in 10 injections at 12 hourly intervals.

Section of the uterus showed good proliferation, while the reaction of the uterus to pituitrin was almost absent. The residual tissue, after the above alcohol extraction, was dried in an air oven, and extracted with ether; the ether was removed under reduced/



reduced pressure and the residue extracted with ethyl acetate. On evaporating the ethyl acetate, a dark brown oil was obtained, similar in appearance to the standard extracts.

Ra 14 received one quarter of this extract beta 13B . The progestational reaction in this animal was completely absent, but the reaction of the uterus to pituitrin was inhibited. Thus by keeping the alcohol and ether extractions separate in the original method of extraction, a partial separation was effected. This was repeated in extract 18, but a similar sharp differentiation was not obtained; the two fractions were therefore combined and beta 18 was considered as a standard extract. It seems probable that the amounts of active material extracted by the ether and alcohol respectively may vary in different batches of material.

It was thought that separation of the two factors (should there be two) might be easier if the crude extracts were purified somewhat. Therefore following the method described by Allen a portion of Beta 18 was treated as follows:- 4 c.c. Beta 18 was dissolved in the minimum quantity of ether and the phosphatides precipitated with 4 volumes of acetone. The ether-acetone solution was filtered off/

off, the phosphatides dissolved in ether and again precipitated with acetone. This was repeated four or five times. The ether-acetone mixture was distilled in vacuo, and the residue dissolved in boiling methyl alcohol and placed in the ice-chest for 12 hours. A large amount of fat and cholesterol separated which was again dissolved in boiling methyl alcohol and placed in the ice-chest. This process was repeated until the fat which separated was lemon yellow in colour and the cholesterol white. The methyl alcohol was removed, and the process repeated. On evaporating the methyl alcohol, a gum-like residue was obtained (the bulk had been reduced to about one quarter of the original extract), and was injected into Ra 128.

The treatment had apparently destroyed the inhibitory potency since the uterus in vitro gave a marked contraction on the addition of pituitrin. Section of the uterus showed good progestational proliferation.

From these few preliminary experiments it will be seen that extracts have been obtained which produce (a) proliferation and inhibition, (b) proliferation without inhibition and (c) inhibition without proliferation. Before it could be stated definitely/

definitely that two factors actually exist, it became necessary to produce extracts which regularly produced one phenomenon or the other and that only. An attempt was therefore made to produce such extracts by partitioning the crude extract between two volatile solvents.

Distribution between 50% ethyl alcohol and petroleum 40-60%.

2 c.c. Beta 12 were dissolved in 10 c.c. petroleum ether and extracted in a separating funnel with 10 c.c. 50% ethyl alcohol. An emulsion was formed and therefore to aid separation one drop of concentrated hydrochloric acid was added. Separation then occurred fairly rapidly and each solvent was thoroughly extracted with the other - about 10 times each. The solvents were then distilled in a good vacuum and the residues dissolved in maize oil and tested. The following table shows the effects of the two fractions.

Table II/

Table II.

Beta	Ra	50% Alcohol		Ra	40-60 Petroleum ether.	
		Prolifer- ation.	Inhibition		Prolifer- ation.	Inhib- ition
12	36	+	++	39	++	-
12		lost		46	++	-
16	65	+++	++	63	+++	-
16	67	+++	++	66	+++	+
16	70	+++	++	69	+++	-

Inhibition	++	complete.	Proliferation	+	slight.
	+	partial.		++	fair.
				+++	good.

It can be concluded then that the corpus luteum extracts under investigation contain two factors, one responsible for the production of proliferation in the uterine endometrium, and the other responsible for the inhibition of the reaction of the uterus to pituitrin.

From Table III it can be seen that the partition coefficient of the proliferative factor between 50% alcohol and 40/60 petroleum ether is about one half, whereas that for the inhibitory factor is nearly one, in favour of the 50% alcohol. A further attempt was made to separate the proliferative/



proliferative and inhibitory factors as follows. The crude extract was dissolved in a small amount of ether and the phosphatides removed by precipitation with 4 volumes of acetone. The acetone soluble fraction, after removal of the solvent, was dissolved in petroleum ether and the solution extracted with 50% ethyl alcohol as described above. The results obtained with this method are shown in Table III.

Table III.

Beta	Ra	50% Alcohol		Ra	Petroleum ether	
		Prolifer- ation.	Inhibition		Prolifer- ation.	Inhib- ition
14	49	+++	-	47	-	-
14	50	+++	+	48	+	++
14	56	+++	+	54	+++	+
17	81	+	-	82	+++	++
17	84	++	++	85	++	+

Proliferation, +++ = good.      Inhibition, ++ = complete  
                               ++ = fair.                               + = partial.  
                               + = slight.

In the second separation of Beta 17, instead of using 40/60 petroleum ether, 100/120 fraction was used. It is obvious however that the removal of the phosphatides prior to partitioning the extract affects/

affects the separation in such a way that no dependable results are obtainable.

At this stage it might be well to illustrate the results so far obtained by means of photographs of the uteri of injected animals and of the records of uterine contractions.



Plate 5. Section of uterus of rabbit (Ra 63) injected  
with petroleum ether extract ( $\beta$ 16) showing  
proliferation +++

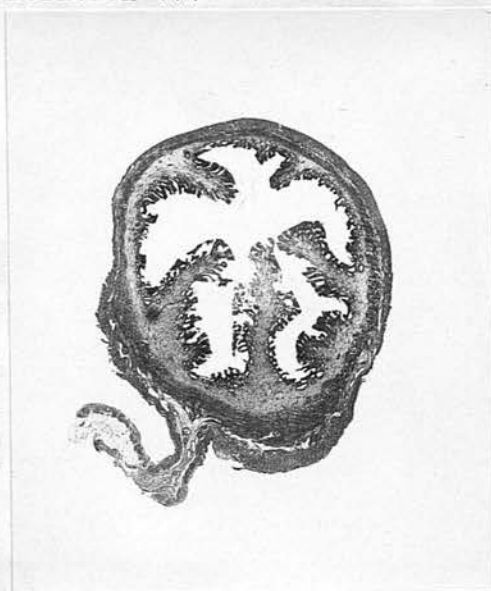
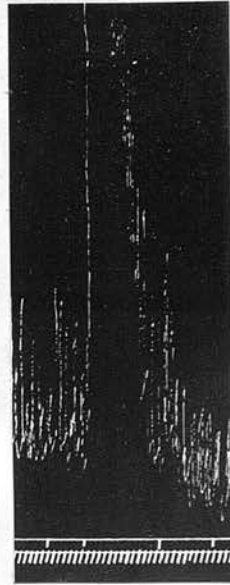


Plate 6. Section of uterus of rabbit (Ra 67) injected  
with 50% alcohol fraction ( $\beta$ 16) showing  
proliferation proliferation +++



7



8

Plate 7. Record of uterine contractions of rabbit (Ra 63)  
injected with petroleum ether fraction ( $\beta 16$ ).

First signal: 5 units pituitrin added to the solution. Time interval = 1 minute.

Plate 8. Record of uterine contractions of rabbit (Ra 67)  
injected with 50% alcohol fraction ( $\beta 16$ ).

First signal: 5 units pituitrin added to the solution. Second signal: 0.1 mgrm. adrenaline added to the solution. Time interval = 1 minute.





Plate 9. Section of uterus of rabbit (Ra 14) injected with ether fraction ( $\beta 13B$ ) showing no proliferation.



Plate 10. Section of uterus of rabbit (Ra 43) injected with alcohol fraction ( $\beta 13A$ ) showing proliferation +++



11.



12.

Plate 11. Record of uterine contractions of rabbit (Ra 14) injected with ether fraction ( $\beta 13B$ ) ..

First signal: 5 units pituitrin added to the solution. Second signal: 0.1 mgrm. adrenaline added to the solution. Time interval = 1 minute.

Plate 12. Record of uterine contractions of rabbit (Ra 43) injected with alcohol fraction ( $\beta 13A$ ).

First signal: 5 units pituitrin added to the solution. Time interval = 1 minute.

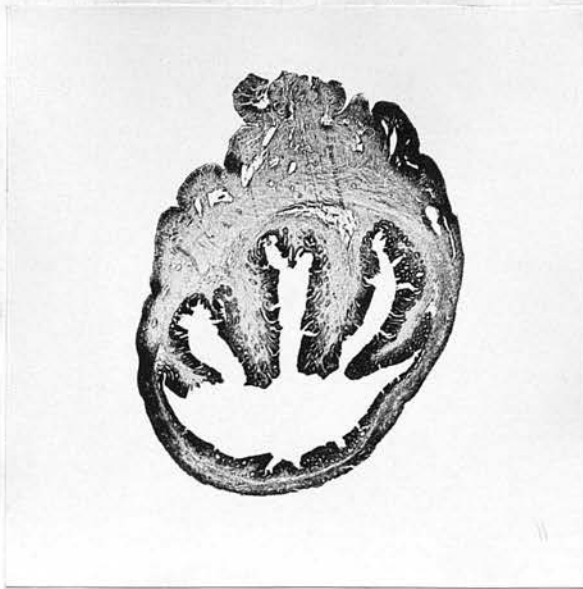


Plate 13. Section of the uterus of rabbit (Ra 27) injected with Beta 10 - 1200 m.u. alpha, showing proliferation ++

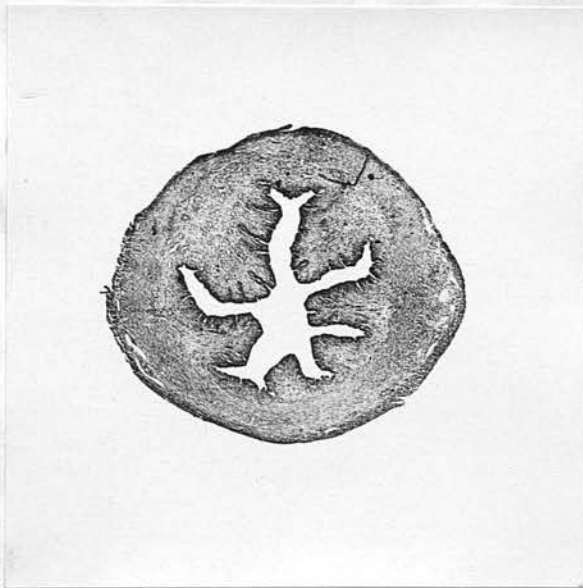


Plate 14. Section of the uterus of rabbit (Ra 35) injected with Beta 12 - 3600m.u. alpha, showing muscular hypertrophy but no proliferation.

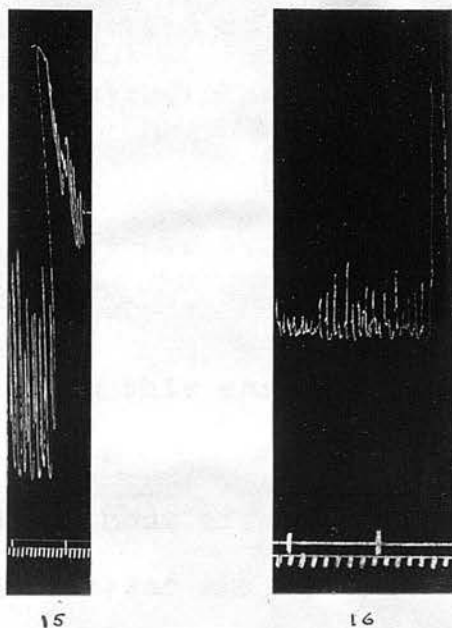


Plate 15. Record of uterine contractions of rabbit (Ra 27)  
injected with Beta 10 - 1200 m.u. alpha.

First signal: 5 units pituitrin added to the  
solution. Second signal: 0.1 mgrm. adrenaline  
added to the solution. Time interval = 1 minute.

Plate 16. Record of uterine contractions of rabbit (Ra 35)  
injected with Beta 12 - 3600 m.u. alpha.

First signal: 5 units pituitrin added to the  
solution. Second signal: 0.1 mgrm. adrenaline  
added to the solution. Time interval = 1 minute.



A number of experiments were then started, varying the concentration of alcohol, and in place of petroleum ether using other organic solvents.

(1) Partition Beta 16 between 45% ethyl alcohol and petroleum ether.

Separation in this case was extremely tedious and the addition of a drop of concentrated hydrochloric acid was without effect. The alcohol fraction was water clear and on evaporation gave a very small amount of solid. The petroleum ether fraction on the other hand was not appreciably reduced in bulk, and on injection into Ra 60 showed marked progestational proliferation and complete inhibition of the pituitrin reaction. No separation was thus effected.

(2) Partition Beta 16 between 50% ethyl alcohol and chloroform.

In this experiment the alcohol soluble fraction when evaporated to dryness was found to be insoluble in ether, by which it was precipitated from solution in/

in aqueous alcohol. This fraction showed no activity whatsoever, while the chloroform fraction caused good proliferation and complete inhibition. These extracts were injected into Ra 73 and Ra 74, respectively.

(3) Partition Beta 20 between 70% ethyl alcohol and 40/60 petroleum ether.

(The original extract Beta 20 showed good proliferation but incomplete inhibition as may be seen by reference to Table I).

The alcohol fraction in this experiment, when injected into Ra 117, showed proliferation of the mucosa ++ to +++ and very slight inhibition. It was not considered worth while injecting the petroleum ether fraction.

(4) Partition Beta 20 between 50% ethyl alcohol and benzene.

Separation was again very slow owing to the formation of an emulsion. The alcohol fraction was water soluble and was injected into Ra 121 in which only very slight proliferation was observable. The benzene fraction was not tested since it was assumed that the active principles had remained in the latter solvent.

(5) /



(5) Partition between 66% acetone and petroleum ether.

Beta 16 was dissolved in petroleum ether and an equal volume of 50% acetone added. On shaking an emulsion was formed which even on addition of acid would not separate. The concentration of acetone was therefore increased to 66%, when separation took place readily. The acetone fraction, insoluble in ether but freely soluble in aqueous alcohol was dissolved in maize oil and injected into Ra 79. Neither proliferation nor inhibition was observed.

The petroleum ether fraction was injected into Ra 80 and resulted in marked proliferation with complete inhibition.

(6) Partition between 66% acetic acid and petroleum ether.

In a preliminary experiment Beta 18 was dissolved in petroleum ether and extracted thoroughly with 66% acetic acid. Injection of the acetic acid fraction into Ra 93 gave good proliferation and complete inhibition.

While no separation was effected in this case, the volume was reduced considerably, and a water soluble extract produced. The weight of the original extract was about 2.5 gm. and that of the acetic/

acetic acid fraction 0.18 gm. It was therefore decided to use three times the amount of extract and attempt experiments in dialysis.

(7) 7 gm. Beta 18 were dissolved in petroleum ether and a like quantity of 66% acetic acid added. Separation was fairly rapid and the petroleum ether was extracted 10 times with a view to extracting as much as possible from the petroleum ether. The combined acetic acid extracts were extracted twice with small volumes of petroleum ether and evaporated under reduced pressure. The residue which weighed 0.35 gm. was divided into three portions: (a) for an ordinary test, (b) for dialysis, and (c) for an experiment over 12 hours in which the extract dissolved in Ringer-Locke would be injected intravenously at 2 hourly intervals.

The first fraction was injected into Ra 96, Section of the uterus showed marked proliferation, while there was a large contraction on adding 5 units of pituitrin to the bath. The second fraction was dialysed through a collodion thimble in distilled water for 4 hours, the water being changed hourly. The dialysate was evaporated in a high vacuum and injected into Ra 102. Neither inhibition/



inhibition nor proliferation was exhibited. The third fraction was discarded. One third of the petroleum ether fraction was injected into Ra 103 and was found to be entirely inactive. This experiment was repeated using Beta 20. One third of the acetic acid extract was injected into Ra 107; fairly good proliferation and partial inhibition resulted. The dialysate was completely inactive, Ra 112, and the non-dialysable part injected into Ra 113 was also inactive.

(8) Partition between methyl alcohol and petroleum ether.

2 c.c. Beta 23 were dissolved in petroleum ether and the solution extracted 10 times with methyl alcohol, i.e. until the methyl alcohol remained colourless. The methyl alcohol was removed in vacuo and the residue dissolved in maize oil and injected into Ra 149. A marked contraction followed the addition of pituitrin to the uterus in vitro, although section of the uterus showed good proliferation. Injection of the petroleum ether fraction into Ra 152 proved it to be inactive. This was repeated with a like quantity of Beta 23 and injected into Ra 159. The results/

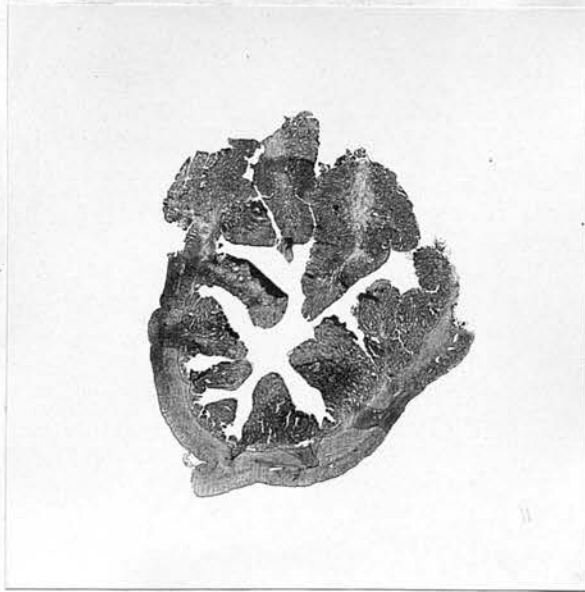


Plate 17. Section of uterus of rabbit (Ra 93) injected  
with acetic acid fraction ( $\beta$ 16) showing  
proliferation +++



Plate 18. Section of uterus of rabbit (Ra 149) injected  
with methyl alcohol fraction ( $\beta$ 23) showing  
proliferation +++

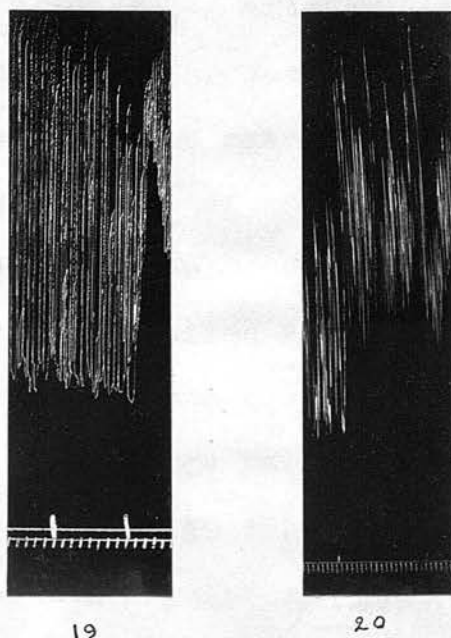


Plate 19. Record of uterine contractions of rabbit (Ra 93)  
injected with acetic acid fraction ( $\beta$  16).

First signal: 5 units pituitrin added to the  
solution. Second signal: 0.1 mgrm adrenaline  
added to the solution. Time interval = 1 minute.

Plate 20. Record of uterine contractions of rabbit (Ra 149)  
injected with methyl alcohol fraction ( $\beta$  23).

First signal: 5 units pituitrin added to the  
solution. Time interval = 1 minute.

results were confirmed.

Since the original extract Beta 23 showed incomplete inhibition, it was decided to repeat this separation using an extract which would inhibit completely the reaction of the uterus to pituitrin. 4 c.c. Beta 24 were treated as described above and the methyl alcohol fraction injected into Ra 164. Good proliferation of the mucosa resulted, but only partial inhibition of the pituitrin reaction.

About this time it was found extremely difficult to obtain regular supplies of ovaries from Glasgow, and those that were received, were poor in corpora lutea. Consequently some considerable time elapsed until sufficient corpora were collected to carry through a preparation of the standard extract.

Beta 29 was such an extract. 875 gm. of corpora were collected during two months, the corpora being stored in 95% alcohol. The preparation was carried out in the normal manner, but on testing the extract on Ra 224 and again with double the dose on Ra 230, it was found to produce/



produce only fair proliferation and the reaction of the uterus to pituitrin was unaffected.

Following this shortage of ovaries, supplies of corpora were received from Holland preserved in acetone. Two batches were received and were dealt with immediately, one of 1550 gm. and a second of 2350 gm. The acetone was drained off the corpora which were then minced and extracted with alcohol as in the preparation of the standard extracts. The acetone and alcohol extracts were mixed, and the solvents removed under reduced pressure. The residue was extracted with ether and the ether extract combined with that obtained from the dried tissue. The ether was evaporated and the residue extracted with ethyl acetate. The acetate soluble portion constituted Beta 26 and 27 respectively. Both extracts were found to be inactive on Ra 204 and Ra 211. On injection of Beta 26 into oophorectomised mice cornification of the vaginal epithelium was produced, thus showing contamination of the extract with alpha.

On enquiry being made in Holland, it was found that ovaries had been preserved in acetone which had been discarded. The corpora lutea had then been dissected out and forwarded to this laboratory in acetone; no precautions had been taken/

taken to remove any cystic fluid. It is probable then that the preserving acetone had removed the active principles and that the contamination of alpha was due to the presence of cystic fluid, since, as reported earlier if care is taken to remove cystic fluid, alpha is not found in extracts of the corpus luteum.

The effect of acetone extraction was then investigated as follows:-

Beta 31. 500 gm. corpora lutea were extracted twice in the cold with 2 volumes acetone for 24 hours each extraction. The acetone was filtered off and distilled in vacuo at a temperature below 45°C. The residue was extracted with ether and constituted Beta 31A. This extract when injected into Rb 3 produced marked proliferation, +++, but the inhibitory reaction was absent.

The residue from the acetone extraction was then treated as in the preparation of the standard extract, i.e. it was extracted with alcohol, ether and ethyl acetate, the ethyl acetate soluble fraction on evaporation of the solvent yielded Beta 31B. On testing this fraction in Rb 4, it also was found to be lacking in the inhibitory factor, while the uterus exhibited fair proliferation, ++.

This/

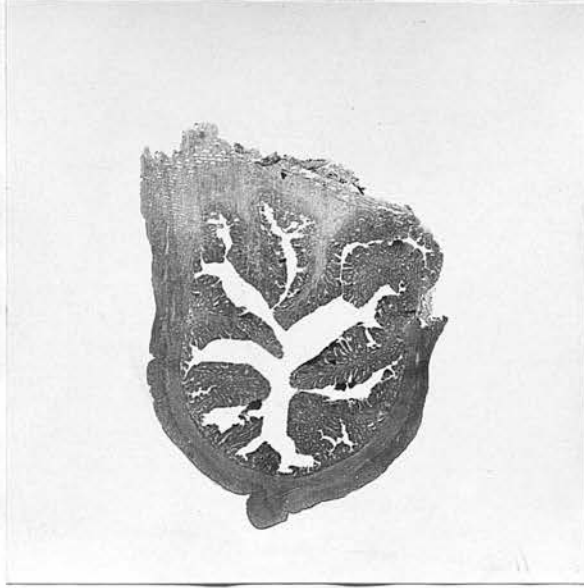


Plate 21. Section of uterus of rabbit (Rb 3) injected  
with acetone extract ( $\beta$  31A) showing  
proliferation +++



22

Plate 22. Record of uterine contractions of rabbit (Rb 3)  
injected with acetone extract ( $\beta$  31A).

First signal: 5 units pituitrin added to the  
solution. Time interval = 1 minute.

This extraction was repeated with another batch of corpora lutea and the results confirmed. Acetone extract Beta 33A injected into Rb 5 - a large contraction resulted on adding 5 units pituitrin to the bath, and section of the uterus showed good proliferation, +++. The residue Beta 33B was injected into Rb 6, and proved to be almost inactive - there being only very slight proliferation, +.

Modifications of the original mode of extraction were now carried out and will be described herewith. Claus (22) describes the separation of gonadotropic hormones from the anterior lobe of the pituitary, and the preparation of one of them in crystalline form. It was decided therefore to apply that author's method to the preparation of a corpus luteum extract, in a modified form. The method adopted is best illustrated by the following scheme:

Corpora/





extract might be examined, not only for the factors of the corpus luteum, but also for gonadotropic factors. Injections were spread over 3 days and 12 hours after the last injection, the animal was killed by a blow on the occiput.

Examination of the ovaries revealed no effect whatsoever; section of the uterus showed no progestational proliferation, and on addition of pituitrin to the uterus in vitro there was a large contraction. The crystals were thus quite inactive. The residue, Beta 28B, was injected into Rb2 which had been treated in the same way as that used for the above experiment. This also proved to be lacking in activity.

Beta 32. 150 gm. corpora lutea were extracted with 2 volumes of ethyl alcohol acidified with hydrochloric acid (2 c.c. HCl + 98 c.c. 95% alcohol) three times for 24 hours each extraction. The alcohol was filtered off and removed under reduced pressure. The residue was extracted with ether, the ether evaporated and the resulting watery solution shaken up with maize oil and injected in 6 doses during 3 days into Rb 8. Section of the uterus showed that marked proliferation had been produced, whereas there was a good response to pituitrin.

The/

The tissue from the above extraction was dried in a current of warm air and extracted with ether in a Soxhlet apparatus, the ether removed in vacuo, and the residue extracted with ethyl acetate. On evaporation of the ethyl acetate, a very small amount of straw coloured gum was obtained, which was injected into Rb 10 and proved to be inactive.

Beta 34. 300 gm. corpora lutea were extracted with methyl alcohol twice for 24 hours each extraction. The methyl alcohol was filtered off and distilled under reduced pressure. The residue which was completely soluble in ether was injected into Rb 7 and gave a fairly good proliferative reaction and complete inhibition.

The residual tissue was extracted with ether, the ether evaporated and the residue extracted with ethyl acetate. As would be expected, when injected into Rb 9, this extract proved to be inactive.

The effect of saponification of the standard corpus luteum extract on its proliferative and inhibitory action was also investigated. Six experiments were carried out as follows:-

(1) /

(1) 2 c.c. Beta 12 were dissolved in 0.5% alcoholic potassium hydroxide and heated for 2 hours in a partial vacuum at a temperature of about 50°C. The alcohol was evaporated under reduced pressure, 30 c.c. of distilled water added, and carbon dioxide passed into this solution for 1 hour. It was then extracted 5 times with ether, the ether evaporated and the residue, dissolved in maize oil, injected into Ra 26. Section of the uterus showed that no proliferation had been produced, while the inhibition of the reaction to pituitrin was incomplete.

(2) 2 c.c. Beta were treated in exactly the same way and the residue injected into Ra 40. In this case there was fair proliferation with inhibition.

(3) 6 c.c. Beta 14 were treated as above, and half the residue injected into Ra 52. The uterus exhibited good proliferation and no inhibition. The other half was injected into Ra 53 and this result confirmed,

(4), (5), (6). In each of these experiments 2 c.c.

portions of Beta 16 were dissolved in 100 c.c. 2% alcoholic KOH and saponified under ordinary pressure on the steam bath. The solution after neutralisation with/



with carbon dioxide, was extracted with ether, the ether evaporated and the residues injected into Ra 60, Ra 61 and Ra 62 respectively. In no case was there either proliferation or inhibition. Little evidence could thus be obtained that saponification under reduced pressure at 50°C. exerted any selective action on either of the two effects under investigation. Saponification of the crude extract under normal pressure destroyed both factors.

Early in this research an attempt was made to remove the fats by acid hydrolysis and subsequent formation of the calcium soaps. The method was as follows:-

The remains of extracts Beta 5-9 were combined and 4 c.c. of the mixture dissolved in 120 c.c. of 95% alcohol. A rapid stream of dry hydrochloric acid gas was passed in for 20 minutes and the solution shaken thoroughly. The solution warmed up considerably and assumed a greenish brown colour. On cooling a saturated alcoholic solution (20 c.c.) of calcium chloride was added and the mixture shaken at intervals during three days. A heavy oil separated and the supernatant liquid was removed. The oil was shaken up with alcohol and the alcohol distilled off in vacuo at a temperature of about 40°C. The/

The residue was extracted with ether, the ether removed under reduced pressure and the brownish gum extracted with ethyl acetate. On evaporation of the ethyl acetate a thick viscous oil was obtained, having a volume of about 1.5 c.c. Half of this was dissolved in maize oil and injected into Ra 23, in which it produced marked progestational proliferation and complete inhibition of the reaction of the uterus to pituitrin.

In the majority of experiments 2 c.c. of the crude extract were used. In those experiments where partition of the extract was made between two solvents the two fractions were injected into oophorectomised rabbits over a period of 3 days at 12 hourly intervals, one pair of rabbits receiving the fractions from 2 c.c. of the original extract.

In one experiment where the extract was partitioned between 50% alcohol and petroleum ether, hydrochloric acid was not added. It was found, on injecting the fractions obtained, that the petroleum ether fraction caused both proliferation and inhibition, while the 50% alcohol fraction caused only proliferation, (Ra 41 and Ra 42).

This /

This result suggests the possible chemical nature of one of the factors which will be discussed later.

In view of the fact that the presence of a number of hormones has been demonstrated in the human placenta, experiments were made to determine whether an extract of the latter would produce any proliferative or inhibitory reaction. The mode of preparation was exactly the same as that used in the preparation of the standard corpus luteum extract. Placentae were received from the Maternity Hospital as fresh as possible, and the preparation carried through immediately with quantities of 700 to 900 gm. Each animal received the whole extract. Five such experiments were carried out and the results obtained are shown in Table IV.

Table IV.

Animal Ra	Wt. of placenta in gm.	Proliferation	Inhibition
18	700	----	++
33	700	----	+
51	850	----	++
55	900	----	----
68	850	----	----

No effect ----

Partial inhibition +

Complete inhibition ++

Three/

Three further experiments were carried out.

(1) A 5 months sheep's placenta was obtained and an extract prepared. This was injected into Ra 86, in which the only observable effect was an alpha one.

(2) 250 gm. human placenta was obtained from an embryo 24 weeks old, and an extract made. This was dissolved in maize oil and injected into Ra 123, in which there was no effect.

(3) The placenta in this case was obtained from a human embryo of 14 weeks. This extract also proved to be inactive (Ra 139).

It will be seen that in no instance was any proliferation induced, while definite evidence of an inhibitory reaction was obtained in three out of five animals.

The similarity in the appearance of the tissue of the suprarenal cortex to that of the corpus luteum suggested the possibility of an extract being obtained which would simulate the corpus luteum extract, and experiments were therefore made to determine if this were actually the case. Suprarenals, obtained from the/



the Glasgow abattoirs shortly after the animals had been killed, were minced and extracted with alcohol, as in the preparation of the standard extracts. In most cases a very large amount of fatty material separated from ethyl acetate, and the bulk of the final extract was comparatively small, - about 20 c.c. per kilo of tissue. In none of the five experiments was there any inhibition, while in four there was some degree of proliferation, and in one marked proliferation. The results are shown in Table V.

Table V.

Animal Ra	Wt. of tissue in gm.	Proliferation	Inhibition
38	500	+	---
78	500	+++	---
92	1100	+	---
218	1000	+	---
231	900	+	---

+ Slight proliferation.    --- No inhibition  
+++ Good do.

Englehart ( 36 ) reports that "the injection of lipoid extracts of suprarenal cortex into young female rabbits causes growth of the uterine mucosa and/

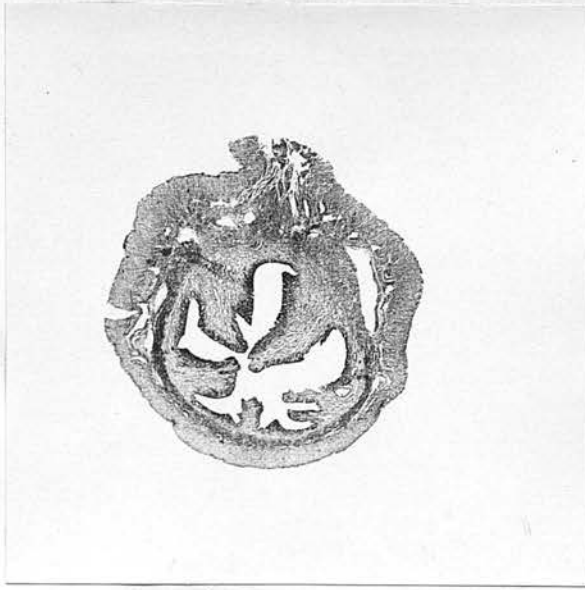


Plate 23. Section of uterus of rabbit (Ra 51) injected  
with placental extract showing no proliferation.

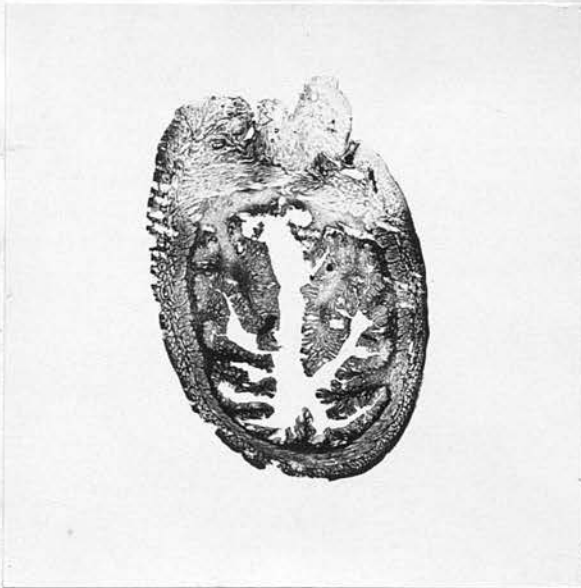


Plate 24. Section of uterus of rabbit (Ra 78) injected  
with suprarenal extract showing proliferation +++

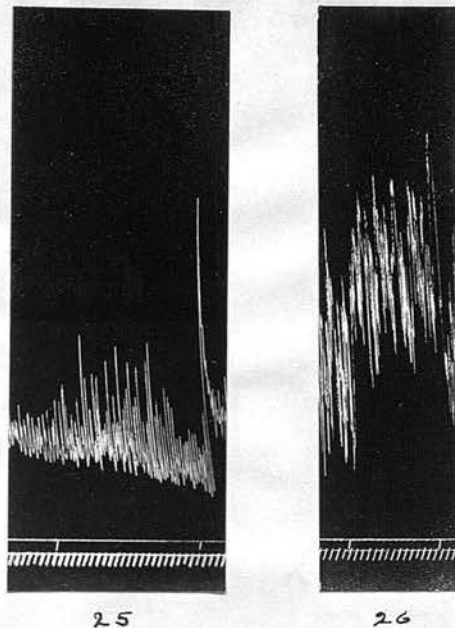


Plate 25. Record of uterine contractions of rabbit (Ra 51) injected with placental extract.

First signal: 5 units pituitrin added to the solution. Second signal: 0.1 mgrm. adrenaline added to the solution. Time interval = 1 minute.

Plate 26. Record of uterine contractions of rabbit (Ra 78) injected with suprarenal extract.

First signal: 5 units pituitrin added to the solution. Time interval = 1 minute.

and musculature". This series of experiments thus confirms the above author's findings.

Two further extractions were made, one of liver and the other of ovarian tissue after removal of corpora lutea and cystic fluid.

(1). Liver. 900 gm. of fresh minced liver was extracted as in the preparation of the corpus luteum extracts, the residue after removal of the ethyl acetate was dissolved in maize oil and injected into an oophorectomised rabbit (Ra 24). Section of the uterus revealed marked muscular hypertrophy, but no glandular proliferation. The inhibitory reaction was absent.

(2) Ovarian Residue. Ovaries were freed from corpora lutea and follicular fluid removed in the press. The minced tissue, weighing 350 gm., was extracted twice in the cold with 95% ethyl alcohol, the alcohol filtered off and distilled in vacuo. The residue was extracted with ether and combined with the ether extract from the dried tissue. The ether was removed and the ethereal residue extracted with ethyl acetate. On evaporation of this solvent a residue/



residue was obtained similar in appearance to the normal corpus luteum extract. This was dissolved in maize oil and injected into Ra 148. On addition of 5 units of pituitrin to the bath, a large contraction followed, and section of the uterus had an appearance of deciduomata formation.

J.J.M. Shaw ( 97 ) suggests "that an excess of cholesterol in the endometrium determines decidua formation"....."that the presence of this substance in menstrual blood" enables the ovum to embed and develop.

Allen in purifying his extracts removed as much as possible of the cholesterol, and if his extracts contained any, there was so little that it gave no colour in the Libermann-Burchard reaction. Yet by injection of such extracts Allen was able to maintain pregnancy in a rabbit oophorectomised 18 hours after mating.

In order to ascertain whether the administration of cholesterol would produce any effect on the uterus, 0.5 gm. of cholesterol was dissolved in 10 c.c. maize oil, and injected into Ra 44, in ten 12 hourly injections. Section of the uterus showed absolutely no effect, and there was a marked response to pituitrin.

It/

It was shown by Reynolds ( 93 ) that the uterus of the rabbit reacts to both the oxytocic and pressor fractions of pituitrin. Throughout this research the response of the uteri of injected animals to pitocin and pitressin was therefore investigated. It was found, as would be expected, that the injection of corpus luteum extracts inhibited the reactions to both the pituitary fractions. In some experiments in which comparatively small doses of corpus luteum extract were injected, it was found that pitocin caused a small motor effect, whereas pitressin was without action or exerted a slight inhibitory effect. There is some indication therefore, that the amount of extract necessary to inhibit the reaction to pitressin was smaller than that needed to inhibit the reaction to pitocin.

The quantities of material used in this research are as follows:-

Corpora lutea.....	20 kilograms.
Placentae .....	4.5 "
Suprarenals.....	4.0 "
Other tissues.....	1.5 "

Chemical/

### Chemical Behaviour.

In only one respect does the extract described in this thesis differ from Allen's Progestin. Allen (3) states that "the oily preparations deteriorate rapidly, but may be kept in ethyl alcohol for long periods of time without loss". Such was not found to be the case during the progress of this work. One extract in particular may be mentioned: Beta 5-9, which was about three months old when tested, was found to have retained its activity completely. The stability of the actual extract to alcohol was not determined, but storage of the corpora lutea in alcohol for long periods was found to destroy the active principles.

Both hormones are soluble in ethyl alcohol, benzene, ether, chloroform and petroleum ether. While Beta is easily soluble in methyl alcohol and acetone, these solvents appear to destroy Gamma,\* if treated with these solvents for any length of time.

Saponification destroys both factors (thus differentiating them chemically from alpha), while acid/

\*The use of the names beta and gamma for the proliferative and inhibitory factors respectively will be justified later (see p. 88).

acid hydrolysis is without effect.

The relationship between Beta and Gamma and those extracts which inhibit oestrus is not yet apparent.

The method of preparation employed for the production of oestrus inhibiting extracts, by Parkes and Bellerby (89), Haterius and Pfiffner (52) and Patel (90) is not very dissimilar from the method described in this thesis. Payne, Cartland and Van Peenan (91) claim to have inhibited oestrus with the non-saponifiable fraction from the corpus luteum; saponification certainly destroys Beta and Gamma. Macht, Stickels and Seckinger use very divergent methods, hence it can only be conjectured that oestrus may be inhibited by non-specific substances.



### III. DISCUSSION.

#### 1. Confirmation of the earlier findings.

The phenomena which have been investigated in this research are the production of progestational proliferation and inhibition of the reaction of the uterus to pituitrin.

The claim of Corner and Allen (25) that corpus luteum extracts prepared by means of organic solvents will produce progestational proliferation when injected into mature oophorectomised rabbits has been supported, and the statement of Knaus (65) that the injection of such extracts into mature non-pregnant rabbits inhibits the in vitro reaction of the uterus to pituitrin, has been confirmed. Both the oxytocic and pressor fractions of the pituitary are inhibited. Thus the rôle of the corpus luteum as an endocrine organ, which both prepares the uterus and takes a part in the control of its motor activity, must be taken as established.

#### 2. The rôle of the corpus luteum.

The analysis of the mechanism controlling the luteal phase of the sex cycle has proceeded sufficiently/

sufficiently far to enable us to ascribe to the corpus luteum definite functions:-

1. Sensitisation and proliferation of the uterine endometrium.
2. Inhibition of the reaction of the uterine musculature to pituitrin.
3. Inhibition of oestrus and ovulation.

It will at once be noticed that the phenomenon of relaxation of the pubic ligaments of the guinea pig has been omitted; as was pointed out earlier the relaxative principle is not found in the corpora lutea of all animals in consequence of which the phenomenon which it produces is not regarded as one of general biological significance. It will be remarked also, that the development of the mammary gland has not been attributed to the corpus luteum. Since Parkes' experiments, which appear to have been insufficiently controlled, suggest that development of the mammary gland is under the sole control of the corpus luteum, and those of Corner, and Stricker and Greuter lead to the conclusion that the hormonal control of lactation is directly under the influence of the anterior lobe of the pituitary, it seems undesirable to discuss the point until further research has been carried out.

It/

It may be assumed that progestational proliferation is the physiological expression of sensitisation, and therefore one function of the corpus luteum is to prepare the uterus for the reception and nutrition of the fertilised ovum. The second function is one of protection: to prevent the uterine musculature from contracting under the influence of the posterior pituitary factors present in the blood.

### 3. One or two factors?

It has been shown that two distinct effects are produced by corpus luteum extracts prepared with volatile media. This presents, as has been pointed out, only a confirmation of previous findings. However, the new result of the experiments carried out by the writer is represented by the fact that some extracts will produce only one or the other of these two phenomena (inhibition and proliferation respectively) although the crude extracts, as a rule, produce both effects. Obviously a question must be asked in view of recent findings of ovarian physiology in general and in view of the present findings in particular/

particular: Can these two distinct effects be referred to the same active principle?

Table VI.

Inhibition	Proliferation		
	-	++	+++
-	7 expts. = 10.3%	9 expts. = 13.2%	13 expts. = 19.5%
++	8 expts. = 11.7%	6 expts. = 9.0%	25 expts. = 36.3%

Inhibition: - = no inhibition.  
++ = inhibition.

Proliferation: - = no proliferation.  
++ = fair proliferation.  
+++ = good proliferation.

This table shows both the histological and in vitro results of tests carried out on 68 animals with the various extracts described. The percentage figures refer to the fraction of the total experiments which gave the particular result under consideration.

It can be seen that in 30 animals, i.e. 44.4%  
either /



either one reaction or the other was obtained; in 31 animals, i.e. 45.3% both reactions resulted, and in the remaining 7 tests the extracts were completely inactive.

In order to facilitate discussion of this question the results obtained in the writer's experiments have been arranged in the Table inserted above. The columns of this table show a number of cases in which no proliferation, slight or fair proliferation and strong proliferation was obtained by means of extracts, and the distribution of the figures in two horizontal lines indicates how many of these cases of varying proliferation were correlated with the occurrence or absence of desensitization for the pituitary reaction. It will readily be seen that this arrangement which follows the method frequently used (see for instance Czuber, "Statistische Forschungsmethoden", p. 19) permits to establish the existence and degree of co-variation of the respective potencies of the extracts (desensitization potency and proliferation potency).

It can be stated definitely that the correlation of the two activities is certainly not an absolute one. This is borne out by the fact that in twenty-two/

two out of sixty-eight cases proliferation was established in the absence of inhibition whereas vice versa inhibition was observed in eight cases in which proliferation was absent.

This must be regarded as the main fact on which to base further discussion. But before entering into the evaluation of this fact, a further examination of the table is advisable. Following again the well-known method, the independency values for the respective combinations have been inserted (in every field of the table). These figures indicate how many cases one would expect to find in any field if the two activities would occur only in chance combinations. The result seems to be very clear. In the first column both actual values are to all practical purposes identical with the values to be expected in chance combinations. In the last column and particularly in the value given for the combination of desensitization with strong proliferation, the actual values are significantly greater than the expected frequency. However, this difference is really misleading. It has to be remembered that the table contains data relating not only to those extracts which were subjected to separation but comprised also the data for crude extracts/

extracts which, in most cases, were chosen because this combination of both activities was present. Therefore, the variation in the second line of the last column between expected and actual frequency has to be omitted from any consideration.

Thus one has to omit from the consideration the figure occurring in the last column in the second line. Consideration of the other figures shows that the combination of the two activities in the extracts used indicates clearly independent variation of the relative strength of the two activities. Obviously this independence of occurrence refers only to the extracts and not to the occurrence of those factors in the corpus luteum itself; though here again a certain independence in the occurrence of the two respective activities is demonstrably shown later.

Generally speaking one can arrive at the following three conclusions: (a) The crude extracts effect both desensitization and proliferation. This of course, is nothing but a repetition of findings made previously; (b) The relative intensity of the two activities varied in the extracts used almost independently of each other; (c) Complete separation of/

of the two activities can be effected.

For the question under discussion, namely, whether one or two factors must be assumed to be present in these extracts, the last case (complete separation of activities) is of greatest importance.

Tausk (103) has confirmed the results obtained, by (a) preparation of crude extracts which exhibit either proliferative or inhibitory activity, (b) by partitioning the crude extract as described herein and obtaining fractions such as have been obtained during the course of this work, and (c) by administration of alpha together with corpus luteum extract in which experiment he confirms the author's results as published previously (96) almost quantitatively.

Robson(95) has shown that the implantation of bovine pituitaries, or the injection of alkaline extracts of anterior lobe substance into a rabbit results in the formation of luteal tissue in the ovary with the corresponding changes in the uterus, and further that the "muscular and endometrial changes may last for different periods in one and the same animal".

It might be argued that these results are due merely to quantitative differences, and that the/



the concentrations of active substance needed to bring about the proliferative and inhibitory reactions are different. This, however, cannot be the case, for, if it be assumed that the amount of substance necessary to cause proliferation is smaller than that needed to bring about inhibition, then the petroleum ether fraction (in those experiments where the crude extract was partitioned between 50% alcohol and petroleum ether) should not only cause proliferation but inhibition also. Similarly the 50% alcohol fraction should cause very marked proliferation in addition to inhibition, if it be assumed that the amount of corpus luteum extract necessary to cause proliferation is greater than that needed to inhibit the reaction of the uterus to pituitrin.

If one assumes that beta and gamma are identical, the results which have been obtained, in that part of the research where inhibitory extracts were obtained from human placenta, might be explained on the grounds that the amount of alpha present in placenta was sufficient to prevent any progestational reaction without interfering with the inhibitory reaction. No estimation of the alpha content of the placenta extracts was made. However it has been/

been stated that the amount of alpha in placenta varies from 200 to 2000 mouse units per kilo placenta, and it is extremely doubtful whether this quantity would be extracted in the experiments described. It has been shown that the injection of as much as 1000 mouse units of alpha scarcely interferes with the proliferative effects of corpus luteum extracts; it seems impossible therefore that the quantity of alpha present in the placental extracts can be responsible for the complete suppression of the proliferative effect if beta were present in the placenta.

It must be assumed therefore that these effects are due to the presence of two active factors in the corpus luteum which have a differential solubility in petroleum ether and 50% alcohol respectively. This conclusion would explain the results obtained with extracts of human placenta, and would offer an explanation for other observations such as the effects obtained by using extracts prepared from corpora lutea by initial extraction with alcohol and ether respectively.

Until it has been ascertained that the two hormones/

hormones perform no other functions than those described, the noncommittal names of beta and gamma will be used, beta being that factor responsible for progestational proliferation, and gamma for the inhibition of the reaction of the uterus to pituitrin.

4. Explanation of pregnancy changes on the beta-gamma theory.

Knaus (64) working on the rabbit carried out very careful experiments on the variation of the spontaneous activity of the uterus during pregnancy, in which any possible effects of enlargement of the muscle fibres on the uterine properties were eliminated by the use of a sterilised cornu containing no foetuses. His results showed that during the first half of pregnancy the uterus is practically inactive owing to the loss of contractility, while during the second half there occurs a continuous rise in spontaneous activity which reaches a climax at parturition. Knaus attributed this behaviour to the changes occurring in the corpus luteum of gestation.

As was pointed out earlier, Knaus produced this "desensitisation" of the uterus to pituitrin in the rabbit by injection of corpus luteum extract and/

and assumed there to be only one hormone. Neither Knaus nor any other author, however, has even attempted to explain, on the basis of this assumption, how it comes about that one phenomenon of the reproductive phase (proliferation) is maintained to the end of the phase, while the other gradually disappears. It is clear that one cannot attribute this difference to a differential threshold (of corpus luteum secretion) for the two phenomena, as has been pointed out before, so that the 'one-hormone' theory is untenable. On the 'beta-gamma' theory however the changes occurring in the uterus during gestation may be readily explained.

That the rate and duration of secretion of beta and gamma may be different is extremely probable, and if it be assumed that the amount of gamma produced is decreased as pregnancy progresses, then the gradual return of the uterine excitability to pituitrin is obvious. Further, it is clear that the termination of gamma secretion prematurely will result in abortion, and it is at least possible, if not entirely probable, that this is the immediate cause of abortion. The function of alpha and its relation to beta and gamma during gestation is still rather obscure. It has already been shown that the injection in the rabbit of small doses of alpha has very/



very little effect on the proliferative reaction, and in large doses overrides the effect of beta; under no circumstances has it been found to have any effect on gamma.

Alpha has been called the oestrus hormone since its injection into oophorectomised animals brings about the phenomenon of oestrus. It might equally well be termed the 'pregnancy' hormone since in many animals the amount of alpha secretion during oestrus is small, whereas during pregnancy the amount excreted in the urine of e.g. man, is relatively enormous.

Little is known as to alpha production in the rabbit during gestation. During the early stages of pregnancy in women, however, when proliferation of the endometrium is essential for the nidation of the ovum, little or no alpha is excreted in the urine. As pregnancy progresses, increasingly large amounts, reaching a climax at parturition, are produced. It is well known that alpha increases the sensitivity of the uterus to oxytocin; it is probable therefore, that the decline of the corpus luteum, and the reassertion of the oestrus producing stimulus, both tend to increase the sensitivity of the/

the uterus to oxytocin. It is difficult to estimate just how important this effect may be in the causation of parturition, but it would appear to be a highly important, if not a crucial, factor.

5. The possible chemical nature of Gamma.

In those experiments in which the crude extract was partitioned between 50% alcohol and 40/60 petroleum ether, the separation of the solvents was greatly hastened if a small quantity of concentrated hydrochloric acid was added, and it was found in the majority of cases, that the petroleum ether fraction caused good proliferation only, whereas the 50% alcohol fraction caused inhibition with varying degrees of proliferation.

If the addition of acid was omitted, the petroleum ether fraction caused proliferation and inhibition, and the 50% alcohol fraction proliferation only.

Now the partition coefficient of hydrochloric acid between 50% alcohol and petroleum ether is nearly 1 in favour of the alcohol, and therefore in those experiments in which acid was added, the alcohol was acid and the petroleum ether neutral or nearly so owing to the low solubility of HCl in petroleum/

petroleum ether and the extremely slight degree of ionisation. It is suggested tentatively that gamma may be basic in character by virtue of which it tends to pass into the acid solvent to form a salt.

Separation of the active principles was not effected, when the phosphatides were removed by acetone precipitation, with any regularity. This may possibly be explained by assuming the existence of a loose absorption compound of gamma with the phosphatides, which is more soluble in 50% alcohol than in petroleum ether. On removing the phosphatides gamma is isolated and is equally soluble in either solvent.

6. The relation of the anterior pituitary to the production of beta and gamma.

Although no conclusive proof has been advanced for the existence of two gonadotropic hormones of the anterior pituitary, it is now generally accepted, at least in the German and American literature, that two really do exist - Rho 1 and Rho 2. Rho 1 is responsible for follicular maturation, while Rho 2 is the kyogenic factor.

As already stated, Robson (95) has obtained the two phenomena, occurring normally during the active/

active luteal phase, dissociated by means of pituitary implants and injection of alkaline extracts of anterior lobe substance.

The question now arises: Does Rho 2 provide the stimulus for the production of both beta and gamma or is a third factor involved, thus:-

Rho 1	.....	alpha production.
Rho 2	.....	beta production.
Rho 3	....	gamma production.

Before this question can be answered, it will be necessary to separate Rho 1 and Rho 2 and prepare them in a state of purity.

7. The occurrence of an inhibitory substance in the placenta.

The fact that inhibitory fractions have been obtained from the corpus luteum of the cow and sow, from an ovarian cyst fluid from a human patient and in some cases from the human placenta, seems to indicate a more general distribution of this factor.

The importance of its occurrence in placenta is not reduced by the fact that not all placentae examined contained an inhibitory fraction. It would be expected that towards the end of pregnancy/



pregnancy the amount of inhibitory substance would be considerably reduced and therefore it will be necessary to investigate further the placenta from the first half of pregnancy. Of course it is by no means certain that the inhibitory fraction of the placenta is identical with the inhibitory factor of the corpus luteum, and it would be inadvisable to draw any conclusions from the experiments with the former as to the nature of the latter.

#### IV. /

#### IV. SUMMARY

1. A method for the preparation of potent extracts of the corpus luteum, which produce progestational proliferation and inhibition of the reaction of the uterus to pituitrin on subcutaneous injection in the rabbit, is described. Both the oxytocic and pressor fractions of the posterior lobe are inhibited.
2. Small doses of alpha in oily solution do not interfere with the progestational or inhibitory reactions of the corpus luteum extracts; large doses of alpha (more than 3000 mouse units) prevent the progestational reaction, but the pituitrin reaction is still inhibited.
3. The crude extract was partitioned between volatile media, and fractions obtained, which produced either one effect or the other. It is suggested that the proliferative and inhibitory reactions may be due to two different factors in the corpus luteum. The names beta and gamma are suggested for the two factors.
- 4./

4. Prolonged treatment of the crude extract with acetone or methyl alcohol destroys gamma without affecting beta.
5. Saponification destroyed both the proliferative and inhibitory actions of the corpus luteum preparations.
6. Inhibition without proliferation was obtained with extracts of human placenta.
7. Proliferation without inhibition was obtained with extracts of suprarenals; thus the statement of Englehart was confirmed.

V. /

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